

Exposure of Cotton Workers in an Experimental Cardroom with Reference to Airborne Endotoxins

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Workers from cotton mills were exposed to cotton dust during carding in an experimental cardroom. Cotton from different geographical locations with varying amounts of endotoxin were used. Exposure levels ranged from 0.6 to 3.6 mg dust/m³ (from a vertical elutriator) and from 0.1 to 8.0 µg/m³ of endotoxin. No relationship was found between the decrease in FEV₁ over the workshift and the amount of airborne dust. Airborne endotoxin correlated with the decrease in FEV₁ and the increase in blood neutrophils. The FEV₁ decrease was more pronounced among smokers.

The data suggest that the amount of airborne endotoxin determines the risk for development of the acute symptoms in the byssinosis syndrome.

Introduction

Persons exposed to cotton or flax dust may develop a series of acute and chronic symptoms, commonly referred to as byssinosis (1). This disease syndrome consists of a subjective chest tightness and an airflow limitation with a slow onset over the shift. These symptoms usually occur on Mondays or on other days after a break in the work. Over the years, the symptoms may be aggravated progressively and be accompanied by signs of chronic bronchitis. Although not included in the classical byssinosis syndrome, mill fever should be considered as part of the syndrome. This is an acute fever which occurs primarily among subjects not previously exposed when they first start working in the mill and which can also be found among workers who experience unusually high exposure levels.

High priority has been placed on identification of the causative agent(s) for the byssinosis syndrome to enable proper protection of the exposed workers. In research to identify the causative agent(s), experimental as well as epidemiological methods have been used. As a part of this work, challenge experiments with persons actively working in cotton mills are important. This presentation will review the experience from a series of such experiments, performed in our experimental cardroom where the importance of smoking habits and bacterial endotoxins (lipopolysaccharide) was investigated. Parts of the results have been published elsewhere (2,3).

Methods

Exposure

The studies were performed in an experimental cardroom, measuring 6.4 × 5.3 × 2.8 m. An old carding machine, without a protecting cover was situated in the middle of the room and cotton laps were processed. The dust levels could be varied by adjusting the ventilation.

In the first series of experiments, middle grade bale cotton was used and in later series cotton from different geographical locations in the United States was used. The latter samples were included to obtain a variation in the amount of endotoxin contaminating the cotton. The exposure in the cardroom was determined as the average value from three vertical elutriators placed on different sides of the carding machine. In addition, subjects carried a personal sampler during the shift.

The elutriator personal sampler filters were weighed to obtain a value for dust levels, and the amount of bacterial endotoxin was determined using the *Limulus* amoebocyte lysate assay (4). The personal exposure to endotoxin was calculated by multiplying the amount of dust from the personal sampler with the amount of endotoxin per mg dust analyzed from the elutriator filters.

Subjects

Workers from two cotton mills were recruited on a voluntary basis and studied on a Monday after an exposure-free weekend. The subjects included both non-smokers and smokers; some had occasionally subjective

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symptoms of byssinosis (grade ½) during their work in the mill, whereas others had no such symptoms. On each test occasion a group of three to five subjects sat and walked in the experimental cardroom where cotton was being carded between 8:00 AM and 2:00 PM.

To assess the importance of endotoxin for the different reactions after cotton dust exposure, subjects were exposed on three different occasions to dust from cottons, each with a different content of endotoxin.

Lung Function Measurements

Before and after carding, the 1-sec forced expiratory volume (FEV_1) was determined by using a Collins survey water spirometer with a computer (Eagle one), basically, according to the recommendations issued by the American Thoracic Society (5). The forced expirations were followed on an XY-recorder. The measurements were supervised by experienced technicians and calibrations were made daily. At least two, and usually several more, satisfactory (i.e., not varying more than 5%) forced expirations were obtained.

The single largest FEV_1 was expressed as percentage of the predicted value, referring to a standard level for subjects with the same age and height. All but three subjects had a baseline FEV_1 in excess of 80% of the predicted value (6). The difference in FEV_1 values before and after the workshift was expressed in percent of predicted, and the average reaction among all workers in a group was calculated and used in the dose-response calculations.

Subjective Symptoms

The subjects were interviewed by using a questionnaire based on the British Medical Research Council questionnaire for byssinosis. The questionnaire also included questions on the presence of cough, chest tightness, airway irritation and fever at the end of the exposure or later in the evening.

Blood Tests

Venous blood samples were drawn before and after exposure. A 10-mL portion of venous blood was drawn into a syringe, and the number of neutrophils and platelets was determined by standard procedures.

Statistical Analysis

For statistical analysis, correlation coefficients were calculated by using the change in individual or group mean FEV_1 values and number of blood neutrophils or platelets as the dependent variable and the dust and endotoxin concentrations as the independent variable after natural logarithmic transformation. All tests were two-tailed and $p < 0.05$ was accepted as statistically significant.

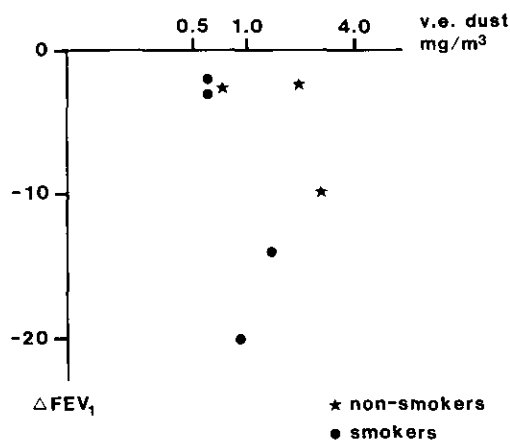


FIGURE 1. Decrease in FEV_1 over workshift for nonsmokers and smokers.

Results

General

A total of twenty experiments are included involving 91 individual observations. The exposure levels ranged from 0.6 to 3.6 mg dust/m³ (from the elutriators) and from 0.1 to 8.0 μg endotoxin/m³. The exposures resulted in subjective symptoms of byssinosis, undistinguishable from those encountered under real working conditions. Individual subjects experienced decreased FEV_1 values as low as 45% compared to the value before shift, and many subjects experienced mill fever and extensive discomfort in the upper respiratory airways at the end of the day.

Influence of Smoking

At the same levels of dust, the FEV_1 decrease over the work shift was more pronounced among smokers than among nonsmokers (Fig. 1). In each group, a few individuals showed a more pronounced reaction than others. However, even if those individuals were excluded, the FEV_1 decrease among smokers was more pronounced than among nonsmokers.

Dust Versus Endotoxin

In the second series of tests, designed to evaluate the importance of endotoxin, there was no correlation between the levels of airborne dust and endotoxin in the different experiments. Thus the exposure conditions were adequate for a separate testing of the individual importance of dust and endotoxin for the observed reactions.

There was no significant correlation between personal sampler dust or vertical elutriator dust, and the individual or mean group changes in FEV_1 over shift. A significant correlation was found between the vertical elutriator or personal sampler endotoxin exposure, and the individual or mean group changes in FEV_1 ($p < 0.01$). Figure 2 reports the relation between the elutriator endotoxin values and the group mean changes

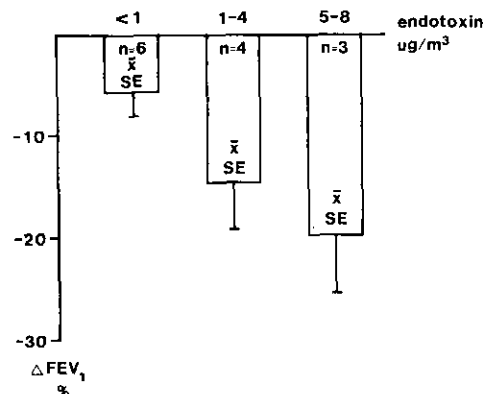


FIGURE 2. Relation between decrease in FEV₁ over shift and airborne vertical elutriator endotoxin levels in three exposure categories.

in FEV₁ over the shift for three classes of endotoxin exposure.

Also in this study, when individual values were studied, it was found that a few individuals showed a more pronounced reaction than the others. The relation between the FEV₁ decrease and dust or endotoxin exposure was not influenced by the exclusion of these subjects.

Blood Cells

The number of blood neutrophils increased over shift in 43 out of 57 observations. Platelets increased over the shift in 25 out of 41 observations.

The correlation between the individual changes in the number of neutrophils and personal sampler endotoxin exposure was significant ($p < 0.05$). The average or individual changes in number of platelets was not correlated to the amount of airborne endotoxin.

There was a significant relationship between the FEV₁ decrease over the shift and the group average increase in neutrophils ($p < 0.01$) as well as between group average FEV₁ decrease and the increase in platelets ($p < 0.05$).

Subjective Symptoms

There was a dose-response relationship between elutriator endotoxin levels and the percentage of persons with subjective symptoms of byssinosis in each group ($p < 0.01$). At the highest endotoxin levels, also some workers without previous symptoms of byssinosis experienced mill fever, pronounced chest tightness, and breathing difficulties at the end of the shift or later.

Discussion

The subjects studied were actively working in cotton mills and not selected for airway reactivity to cotton dust. The population thus differs from that which has been used in other experimental cardroom studies where a significant drop in FEV₁ after a pilot challenge was prerequisite to be included in the panel (7).

The presence of fully developed subjective symptoms

of byssinosis among subjects without a previous history of byssinosis suggests that the cellular mechanisms for the development of byssinosis respond in a toxicological rather than in an immunological/hypersensitivity manner. At high dose levels, symptoms can be made to appear also among persons previously without such symptoms and indeed also in subjects previously never exposed to cotton dust (2).

The more pronounced decreases in FEV₁ among smokers as compared to nonsmokers agrees with earlier studies (8). The reason for this increased reaction is not known. One possible explanation is the increased number of neutrophils present in the respiratory epithelium of smokers (9) with a resulting increased airway sensitivity (10). Alveolar macrophage activation among smokers may also play a role.

In the second series of experiments where the manipulation of the endotoxin content in the carded cotton took place, a high correlation was found between airborne endotoxin levels and the decrease in FEV₁. These findings are in agreement with observations from several previous investigations (7,11,12).

Other previous studies have found a significant correlation between dust levels and FEV₁ changes over the shift as well as subjective symptoms of byssinosis. The reason for the discrepancy between these studies and the present could be that other studies have mainly comprised the working population in one mill, where endotoxin levels in relation to dust levels were the same, or that they were performed in mills where the cotton lint used was fairly similar with regard to the microbiological contamination. This is supported by the results reported here from the first series of experiments where no manipulation of the endotoxin levels was undertaken. In that case a correlation between dust levels and FEV₁ decrease was found (data not shown).

Previously, it has been shown that the number of neutrophils on the nasal respiratory epithelium increases over the shift (2). Neutrophils are activated by endotoxin or gram-negative bacteria on cotton dust and could release mediators for inflammation and bronchoconstriction, such as leukotrienes or platelet activating factor.

The increased number of neutrophils in the airway epithelium could thus be the reason for increased sensitivity to, e.g., methacholine, which has been observed among cardroom workers at the end of the shift (13). The relation between the decrease in FEV₁ and the increase in blood neutrophils agrees with a recent study where the same relationship was found in smokers (10).

The increase in the number of blood neutrophils and its relation to airborne endotoxin agrees with several earlier observations. This increase in the peripheral blood reflects the initial migration of neutrophils into the respiratory epithelium (2,8,14), causing a mobilization of neutrophils from the venous marginal pools. This reaction has also been demonstrated in a dose-dependent manner in animals exposed to cotton dust or endotoxin (15,16).

The relationship between the number of circulating

platelets and the decrease in FEV₁ suggests that platelets may play a role for the symptoms after exposure to cotton dust. The statistical relationship in this study was relatively moderate but must be considered as biologically acceptable in view of the large methodological errors involved in determination of blood platelets. The hypothesis regarding an involvement of platelets in the byssinosis syndrome is supported by previous studies. In one study on cotton workers (17), the number of platelets at the end of the working week was found to be lower than on Mondays before the shift. In animal experiments, an accumulation of platelets in guinea pig lungs (18) and in the lung capillaries of hamsters (19) has been demonstrated after exposure to endotoxin.

Our conclusions are that gravimetric vertical elutriator cotton dust measurements are not related to the biological activity of airborne cotton dust. Our data suggest that the amount of airborne endotoxin determines the risk for the workers to develop the syndrome of byssinosis including mill fever, chest tightness, and acute decrements in FEV₁. This hypothesis is apart from observations of cotton workers carding cotton supported by data from animal experiments. The effects of endotoxin and pure lipopolysaccharide have been extensively studied in animal experiments, involving inhalation. Increases in body temperature, macrophage activation with production of lysosomal enzymes and mediators for bronchoconstriction and platelet accumulation in the lung capillaries causing increased pressure in the pulmonary artery system have been demonstrated (20).

The relationship between endotoxin and the different symptoms in the byssinosis syndrome does not necessarily mean that the pure lipopolysaccharide as such is the sole agent responsible for the effects. Endotoxin could act synergistically with other agents, such as cell wall proteins from the gram-negative bacteria or other particulates in cotton dust.

Conclusion

The results from the experimental cardroom studies made on Mondays on cotton workers exposed to different concentrations of airborne cotton dust with a varying amount of endotoxin, enable us to draw the following conclusions.

The FEV₁ decrease over the shift is more pronounced among smokers than among nonsmokers.

When a systematic variation of the amount of endotoxin in the cotton is undertaken, a significant relationship is present between airborne endotoxin levels and the different symptoms in the byssinosis syndrome. No such relationship is present between dust levels and the presence of symptoms.

The decrease in lung function over the work shift is related to an increase in the number of neutrophils and platelets in the blood.

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